AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

BEST AVAILABLE COPY

STATUTORY DECLARATION

I, Kari Alitalo, a Research Professor of the Finnish Academy of Sciences, at The Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, P.O.B. 63 (Haartmaninkatu 8) 00014 University of Helsinki, Finland, do solemnly and sincerely declare as follows:

I. INTRODUCTION

1.1 In February, 2000, I executed a first statutory declaration (hereinafter referred to as "OKA1" (Opponents, Kari Alitalo, 1st Declaration)) to provide experimental evidence in support of the opposition filed by Ludwig Institute for Cancer Research ("Ludwig Institute") to the issuance of a patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764 ("the opposed application"). That first declaration included a brief summary of my scientific credentials and an introduction in which I set forth some conventional terminology and relevant background information regarding VEGF-C and signal peptides. The first declaration summarized controlled experiments which demonstrated that VEGF2 as taught in the opposed application cannot be expressed and secreted by cells.

1.2 I executed a second statutory declaration (hereinafter "OKA2") in September, 2001. My OKA2 declaration responded to criticisms of the experiments in OKA1

alleged in declarations by Jennifer Ruth Gamble (hereinafter "AJG1"), Nicholas Kim Hayward (hereinafter "ANH1"), and Stuart A. Aaronson (hereinafter "ASA1"), filed on behalf of HGS (OKA2 at 1.2). I repeated and revised the experiments reported in OKA1 in order to address every criticism alleged by HGS in the first group of HGS declarations. The data again showed that cells cannot express and secrete VEGF2 as taught in the opposed application.

1.3 HGS then filed second declarations by Dr. Hayward (hereinafter "ANH2") and Dr. Aaronson (hereinafter "ASA2") critiquing the experiments performed in OKA2. Ludwig Institute asked me to design and perform further protein expression experiments that would address any criticisms raised in ANH2 and ASA2. Further, Ludwig Institute asked me to comment on the data from expression studies performed by Dr. Susan Power and reported in her second declaration (hereinafter "ASP2"), filed at the same time as ANH2 and ASA2.

1.4 I hereby reaffirm my understanding that I have an overriding duty to the Patent Office (and to any Australian Federal Court that should review the Patent Office decision) to provide objective scientific analysis that I believe to be truthful. I hereby affirm that, to the best of my knowledge and belief, factual statements herein are true and opinion statements herein represent my objective scientific opinion and analysis.

II. EXPERIMENTS TO ADDRESS CRITICISMS REGARDING ALLEGED LACK OF CONTROLS, TRANSFECTION EFFICIENCY AND ANALYSIS OF EXPRESSION AND SECRETION OVER MORE THAN ONE TIME POINT

2.1 At the outset, I observe that HGS declarants failed to raise any of these criticisms when commenting on my first declaration. Had they done so, I would have addressed and reported these details in my second declaration. The transfection efficiency and time course experiments relate to rather conventional procedural details, and I am not accustomed to being asked to report them (e.g., when I submit manuscripts of my research to journals for publication).[1] The criticisms alleged in the latest HGS declarations do not cause me to change

[1] The HGS declarants continue to argue that the opposed application teaches to construct expression vectors in which the 350 amino acid VEGF2 sequence is fused in frame with a (heterologous) signal sequence. (See, e.g., ASA2 at 6-8.) I have already explained that my experimental design is based on actual teachings of the opposed application, whereas the experiments now suggested by HGS are not. (See, e.g., OKA at 2.2-2.3 and 5.3.)

my conclusions expressed in my earlier declarations, and I still believe that all of the data from those declarations is sound.

2.2 The following analysis provides evidence that the data provided in OKA1 and OKA2 is accurate and credible, and further supports the position that cells cannot express and secrete VEGF2 as taught in the opposed application.

A. Experimental procedure

1. Cells and Plasmids:

3.1 Results reported in OKA2 revealed that COS and 293T cells were equally appropriate cell lines for analyzing VEGF2 protein expression and secretion. For these new experiments, 293T cells were grown in DMEM supplemented with 10 % fetal bovine serum, glutamine and penicillin/streptomycin.

3.2 The polymerase chain reaction (PCR) was employed to construct a cDNA fragment that corresponded to amino acids 70 to 419 of prepro-VEGF-C. For the purpose of these experiments (directed to assessing transfection efficiency and protein expression at various time points) the cDNA fragment encoding amino acid residues 70 to 419 of prepro-VEGF-C corresponds appropriately with the cDNA encoding the full length sequence of the VEGF2 polypeptide described in the opposed application. Nucleotides 559 to 1608 of the VEGF-C cDNA (Reported in Document D70, Joukov et. al. 1996, GenBank accession number X94216) were PCR amplified with the primers 5′-CGCGGATCCATGACTGTACTCTACCCA-3′ containing a BamHI site and 5′-CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGG-GTACTCGAGGCTCATTTGTGGTCT-3′ containing a XhoI site, HA-tag, a stop codon and a XbaI site and cloned into pcDNA1(Amp)-vector (Invitrogen). The resultant vector was designated as VEGF-2(HGS)/pcDNA1.

3.3 As previously reported in OKA2, an expression vector was also constructed that contained the full length (419 codons) VEGF-C sequence (OKA2 at 3.3.3) for use as a positive control in the expression and secretion analyses. The resultant vector was designated as VEGF-C/pcDNA1.

2. Transfection and time course:

3.4 A principle criticism alleged by the HGS experts was that my OKA2 declaration failed to include transfection efficiency data (ANH2 at 1.5; ASA2 at 24). Thus, for these new experiments, two separate expression vectors, pRL expressing Renilla Luciferase (Promega) and pCMV-ß-gal expressing ß -galactosidase under CMV promoter, were used as transfection controls.

3.5 The other principle criticism of the procedures reported in my OKA2 declaration was regarding the lack of time points in the expression analyses (ANH2 at 1.5; ASA2 at 25). To address this concern three different time points were tested in the new experiments. In particular, the 293T cells were split 1:6 and fresh medium was changed 19 hours thereafter. Three hours after medium change, VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or empty vector were co-transfected with either pRL (three plates with each combination in a 16:1 ratio), or pCMV-ß-gal (one plate with each combination in a 8:1 ratio), using FuGENE6 Transfection Reagent (Roche). The conditioned media and the cells were harvested 24 hours, 48 hours, or 72 hours after the transfection for the purpose of evaluating protein expression and secretion at these different time points. Either twenty-four hours (for time points 48h and 72h) or sixteen hours (for time point 24h) prior to harvesting, the cells were washed twice with PBS and changed to 3 ml of MEM medium containing 100 µCi/ml ^{35}S-methionine and ^{35}S-cysteine (Promix, Amersham) for metabolic labeling of proteins synthesized by the cells[2]. At the indicated time points the conditioned media was harvested and cleared by centrifugation. The cells were trypsinized, washed twice with PBS and lysed in 1X passive lysis buffer from the Dual-Luciferase Reporter Assay System (Promega).

3. Immunoprecipitation:

3.6 Immunoprecipitation experiments were conducted to identify the presence of the various VEGF-C or VEGF2 polypeptides in the conditioned media.

3.7 For immunoprecipitation, 1.25 ml of each conditioned media was supplemented with BSA and Tween 20 to final concentrations of 0.5% and 0.02%, respectively. The different VEGF-C peptides were immunoprecipitated with polyclonal antibodies raised

[2] See explanation in OKA2 at 3.4.2

against a synthetic peptide corresponding to amino acid residues 104-120 of the VEGF-C prepropeptide (Antisera 882, reported in Document D71, Joukov et al., 1997) at 4 °C for 2 hours. This peptide is present in the secreted form of VEGF-C, and the opposed application teaches that it should be present in mature VEGF2 as well. Thus, antisera raised against this peptide should recognize VEGF2 or VEGF-C polypeptides produced by the cells.

3.8 The immunocomplexes were precipitated with protein A-Sepharose for 1 hour and washed 2 times with 1X binding buffer (0.5% BSA, 0.02% Tween20 in PBS) and once with PBS at 4°C. The proteins were analyzed by SDS-PAGE in a 12 % gel under reducing conditions. Half (50%) of each immunoprecipitate sample was loaded into each lane. Since cell cultures contained equivalent amounts of media and equal volumes were used for immunoprecipitation and loading, each lane received an equal sample aliquot for analysis.

4. Luciferase assay:

3.9 The protein concentrations of the cell lysates were determined by the BCA Protein Assay (Pierce) and the luciferase activity in the lysates was measured with the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

5. ß-galactosidase staining:

3.10 The cells were washed twice with PBS, fixed with 0.05% glutaraldehyde in PBS for 15 minutes at room temperature, washed three times with PBS, and stained over night with 2.5 mg/ml X-Gal (5-bromo-4-cloro-3-indolyl-ß-D-galactopyranoside) in 5 mM $C_6FeK_4N_6$ (kaliumferrocyanid) and 5 mM $C_6FeK_3N_6$ (kaliumferricyanid), 2 mM MgCl2, 0.01 % sodium deoxycholate, 0.02 % Nonidet P-40 in 0.1 M phosphate buffer, pH 7.3 at 37°C.

B. Experimental results

4.1 VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or the mock vector were co-transfected into 293T cells with a plasmid encoding the Renilla luciferase gene and the secretion of VEGF-C into the conditioned medium as well as the luciferase activity in the cells were analysed 24, 48, and 72 hours after transfection.

4.2 Similar to the results reported in OKA2, no VEGF2 protein was detected in the conditioned media from the cells transfected with the VEGF-2(HGS)/pcDNA1 construct at

any of the time points tested, over a time period of 72 hours (Exhibit KA-1 Figure, Lanes 1, 4 and 7). In contrast, VEGF-C protein was effectively expressed and secreted by cells transfected with a vector encoding the full length VEGF-C. The different forms of VEGF-C immunoprecipitated from the conditioned medium correspond to partially and fully processed forms of VEGF-C (Exhibit KA-1 Figure, Lanes 2, 5 and 8). These results are in agreement with the results reported in OKA1 and OKA2, and provide evidence that VEGF2 as taught in the opposed application cannot be expressed and secreted by cells.

4.3 To address criticisms suggesting low expression levels of VEGF2 were a result of poor transfection, transfection efficiency was tested in two ways. In one experiment, luciferase activity of the cell lysates was measured and the relative light units/μg of protein were found to be comparable in the cells transfected with VEGF-2(HGS)/pcDNA1 and VEGF-C/pcDNA1. Because the co-transfected luciferase plasmid encodes an enzyme that causes production of light waves under the assay conditions that were used, the measurements of light units provides an indication of the relative transfection efficiency of the cells. In a second experiment, transfection efficiency was also analysed by ß-galactosidase staining of separate plates in which the vectors were co-transfected with a plasmid coding for ß-galactosidase. The ß-galactosidase is an enzyme that causes production of a colorometric product, under the assay conditions used, and thus produces an independent measure of relative transfection efficiency. Results of the transfection efficiency analysis using either of these controls revealed that the vectors were introduced into cells equivalently in the transfections, and thus ruled out the possibility of poor transfection as a cause for the absence or decreased level of VEGF2 protein expression.

4.4 To address criticisms suggesting that the absence of VEGF2 expression was attributable to the length of time that the cells were cultured in the OKA2 experiments, cells were cultured in these experiments for various lengths of time prior to harvesting the cells or media. As the figure shows, cells do not express and secrete VEGF2, no matter what time point is used to terminate the experiment (see Exhibit KA-1). Expression of VEGF-C polypeptides, which serves as a positive control, was visible at all time points studied. Expression was already visible at 24 hours and was strongly visible at 48 hours and 72 hours.

C. Conclusions

4.5 My experiments reported in OKA2 were intended by me to respond to the criticisms raised by HGS in its first series of declarations. Drs. Hayward and Aaronson attempted to discredit the experiments in OKA2 by pointing to alleged new flaws in the experimental design that they failed to raise in their first declarations (ANH2 at 1.5; ASA2 at 5). As reported above, I have now conducted another set of experiments that directly addresses the new criticisms raised in ANH2 and ASA2. The result of those experiments has, once again, revealed that cells cannot express and secrete a VEGF-2 protein as taught in the opposed application.

III. COMMENTS ON EXPERIMENTS PERFORMED BY DR. POWER IN ASP2

A. Comments regarding Dr. Power's experimental Procedures

5.1 In my second declaration I explained that Susan Power's experimental design and results have nothing to do with the teachings in the opposed application. I remain of that opinion. (See. e.g., OKA2 at 2.2-2.3 and 5.3.) The same analysis is true of her second set of experiments reported in ASP2, and I repeat that analysis by reference.

5.2 In my second declaration I explained that Susan Power could not have used the starting materials (ATCC Clone 75698, referred to in the opposed application as amended) that she said that she used in her experiments. (See OKA2 at 5.3.) She has confirmed that my analysis of this issue was correct. (See ASP2 at 5-6.)

B. Comments regarding Dr. Power's experimental results

5.3 In my second declaration I observed that the approximately 30 kDa protein reported in the Power experiments had seemingly no relevance to the opposed application, which makes no mention of this species of polypeptide. (See OKA2 at 5.6.) No explanation was given by Dr. Power in her second declaration to explain the significance of this protein to the opposed application, even though, in her second set of experiments, she again reports "a broad band resolving at approximately 30 kDa." (ASP2 at 31.) In my opinion, to the extent the opposed application teaches anything about protein size, it teaches the 350 amino acid VEGF2, the mature VEGF2 of about 326 amino acids (application at p. 5), and *in vitro*

expression of proteins of 36-38 kD or 38-40 kD (Example 2). There is no mention or suggestion of a 30 kDa band.

5.4 The gels embodying Dr. Power's results deserve one additional comment.

5.5 Although Dr. Power did not describe in detail the volumes in her sample loading in her second declaration, it appears to me that she loaded larger (or more heavily concentrated) samples from some transfection experiments than others. This has had the effect of making the results of interest (the bright colored bands) in different lanes and different gels look identical to each other. The evidence supporting this conclusion is the presence of very black spots that appear between the 50 and 64 kD lane markers in the samples derived from cell supernatant (medium). 5.6 Dr. Power does not explain what these dark spots are, or even acknowledge their existence. In my opinion, since the black spots only appear in the even-numbered "supernatant" lanes, it would appear that the spots reflect a proteinaceous component of the growth medium that Dr. Power was using and that would have been transferred to the gels in the "supernatant" lanes. The likely candidate would be serum albumin proteins that were present in the growth medium used by Dr. Power. (See ASP2. at 22. Dr. Power concentrated the medium (and thus the proteins in it) before running it on her gels. ASP2 at 23.)

5.7 Had Dr. Power loaded comparable amounts of sample in each lane, and in each gel, then I would have expected comparable amounts of serum albumen in each lane, and hence, similar sized black spots (or no spots whatsoever) in all supernatant lanes. However, the black spots have different intensities, suggesting to me that Dr. Power may have loaded different amounts of samples to affect the way that her bright bands appear on the gel. For example, some of Dr. Power's "24 hour" lanes have more prominent dark spots than her 48 hour lanes. (Compare spots in Gel 3, lanes 2, 4, or 6 to Gel 3, lane 8.) Also, the dark spots in Gel 1 appear darker than Gel 2 or 4.) Thus, Dr. Power appears to have been adjusting the size of her samples, so that her "bright spots" would appear the same in her various experimental samples.

IV. CONCLUDING REMARKS

6.1 The protein expression and secretion studies I report herein were designed to address any criticisms made by HGS with regard to experimental design credibility. The

results demonstrate several key points. First, VEGF2 as taught in the opposed application cannot be expressed and secreted by cells. The data clearly establishes the failure of VEGF2 to be expressed and secreted at multiple time points over a period of 72 hours (Each of these time points was sufficient to observe expression and secretion of the VEGF-C positive control run under the same experimental conditions). Second, transfection experiments reported herein rule out the possibility that poor expression of VEGF2 was due to lower amounts of the VEGF2 expression vector being introduced into the cells. Thus, controls, transfection efficiency and expression and secretion over time have all been accounted for in this declaration.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at HELSINKI FINLAND

This 16 day of July, 2002

Kari Alitalo

BEFORE ME:

(Signature of Notary Public)

LAURI HAIKARAINEN
Notary Public

HELSINGIN MAISTRAATTI
MAGISTRATEN I HELSINGFORS

SPRUSON & FERGUSON

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

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THIS IS Exhibit KA-1

referred to in the Statutory Declaration

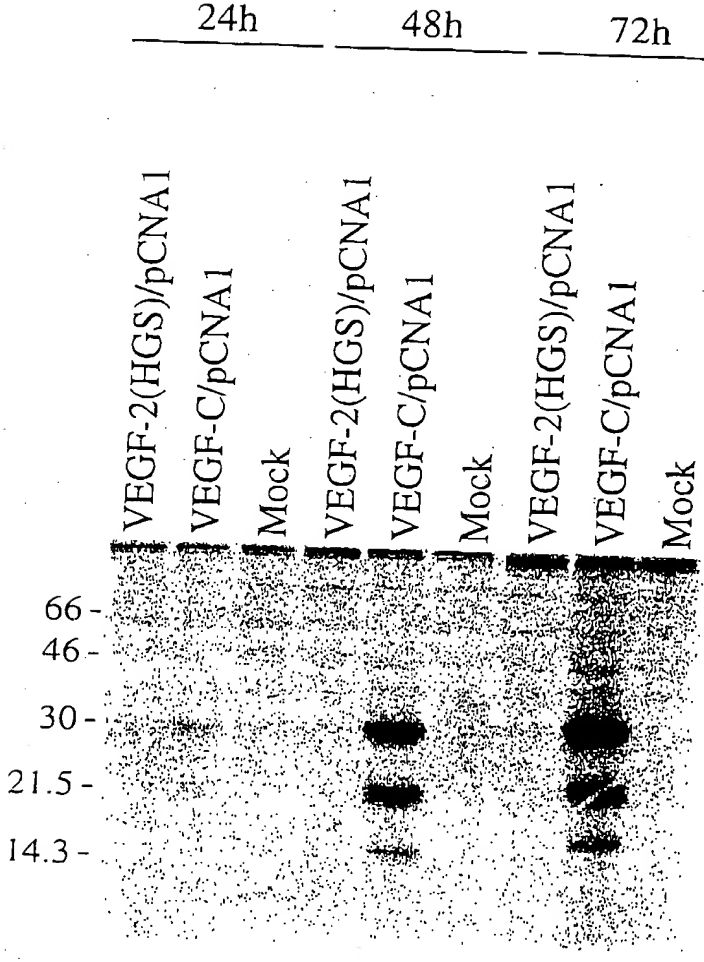
of Kari Alitalo

made before me

DATED this Day of July 2002

(Signature of Notary Public)

SPRUSON & FERGUSON



Figure